Abiotic stress upregulated TaZFP34 represses the expression of type-B response regulator and SHY2 genes and enhances root to shoot ratio in wheat

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Published in:
Plant Science

DOI:
10.1016/j.plantsci.2016.07.011

Publication date:
2016

Citation for published version (APA):
Chang, H., Chen, D., Kam, J., Richardson, T., Drenth, J., Guo, X., McIntyre, C. L., Chai, S., Rae, A. L., & Xue, G-P. (2016). Abiotic stress upregulated TaZFP34 represses the expression of type-B response regulator and SHY2 genes and enhances root to shoot ratio in wheat. *Plant Science*, *252*, 88-102. https://doi.org/10.1016/j.plantsci.2016.07.011

Appendix A. Supplementary data

Abiotic stress upregulated TaZFP34 represses expression of type-B response regulator and *SHY2* genes and enhances root to shoot ratio in wheat

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Supplementary Table S1. The sequences of primer pairs used for real-time PCR.

Gene name	GenBank accession #	Forward primer	Reverse primer
TaZFP22	EU408222	5′-cgtgtgccggaagacgtt	5′-cgtcgtagtggcacctttg
TaZFP34	EU408224	5′-acggcgatcagtgggtgt	5′-gacgaacagctcgagcaaga
TaZFP46	EU650398	5′-acaacgacagcagcgacaat	5′-gtgggtggttcaggtcgag
TaRR12B	AK336252	5′-aagctgcagggtggattcaa	5′-cacacccgaggtcattttcc
TaRR12D	CJ612635	5′-gcagggtgggttcaattcc	5′-ctctcatatgcaggcaccaagt
TaRR1L1	CK206029	5′-cattggaagcagtggagacatc	5′-tccagggattaccctacttacaaatc
TaRR1L2	JF951927	5′-cagccagtgtccagttcgtcta	5′-aagcacccttcccaacaaatc
TaIAA7	AJ575098	5′-caagggccaaggacaagtacaa	5′-caccatccttgtggcatcatag
TaIAA8	GH723801	5′-tgcagctggaatagctccaa	5′-ccatcgacatcatcgcaact
TaIAA27	HX049647	5′-gccgccgagaagagtaagaa	5′-cgatcgcaggaaaggaaaca
TaSHY2	HX177892	5′-ggatcatgaagggatccgaag	5′-catggcatgttgcgtgttct
TaGA20-ox1	FR716525	5′-ggaagtcgctggccttctt	5′-cacgtgaagtccgggtagg
TaGID1	FR668556	5′-caagtacttcgtcacgctccag	5′-ggcaggtacgccttccagta
TaRht1	KC767924	5′-ccaggtcatgtccgaggtgta	5′-ggccaccacgttgcagat
TaARF1	AK331474	5′-tcagactatccagcctcgtttg	5′-cccgaatgatcaaaggaagaaa
TaARF2a	AK334169	5′-ggaaggttcagtggatggtgac	5′-tggctagaatttaaatcgcttgatg
TaARF2b	AK335756	5′-tcaaacaaggattggcagattg	5′-cttgcgcactatgttgcagaac
TaARF6	BJ313449	5′-gcagcagcggaactgtttctat	5′-ggcaaatcttcgccactatacc
TaETTINa	AK330400	5′-atttggcatttctctggctgaa	5′-atagttggcaccaccatcatca
TaETTINb	AY376128	5′-cgacgtccaacaatggatatga	5′-caaatcaagaatcaccaacagatga
TATIR1L1	AK330774	5′-gcctaatctggtggttgaagtga	5′-tgccaatgatcgatacaggtaca
TATIR1L2	CJ589802	5′-ccgccttaccgtggagataat	5′-ggctatcatccggaagtgactc
TaTIR1L3	HX160462	5′-aatgcctaatctggtggttgaag	5′-gccaacgatcgatacaggtaca
TaPIN1a	CK162375	5′-catacatgccagccaactgtg	5′-gccagatgcacacaaatgtca
TaPIN1b	AY496058	5′-ctcagcacggctgtcatcttc	5′-aggccgagcaggatgtagtaga
TaPIN2	BT008949	5′-tgctcctacatgttgccattgt	5′-tgtactccttggcgaacacaaa
TaPIN4	CA703452	5′-gagccaaggagagggaggacta	5′-cctgttcccgaagctgaagtc
TaExpB18	CD897610	5′-cctcgtggcgagtaacgtc	5′-acccaacccacgcctagtt
TaCYCB1-2	AK334336	5′-tccatggagaagaatatcctgaaca	5′-atctcggcgaagaagaagatca
TaCYCB2-3	CJ847821	5′-atgtcctggaacaagtgctgtg	5′-ctgcagtccatgagctgttctt
TaCYCD4-1	AF512432	5′-ggatctccagcaacatgctacc	5′-gatcggtcatctgccaattctc
TaNCED3	LC077864	5’-ccaaggtggacctggtgac	5’-ccctcgccgtactcgaact
TaNCED9	LC077862	5’-gggagctcaccaagttcgag	5’-gtcgtgaacgaaggtgagaatg
TaCYP707A1	LC077861	5’-gctggagatgctcgtcctct	5’-tctcggacttggaggtggac
TaABI1	AB238930	5’-gctgctgaatgcttgtcgaa	5’-cctctgcgccttgaggtcta
TaNAC69-1	AY625682	5’-tgcctcccgaaaaccca	5’-ttgttcacgtagccgttgttgt
TaWRAB1	AF139915	5′-aatgccaccaccaaggacac	5′-gcgaacgatgcgtctagtga
TaWRAB18	AB115914	5′-aggacagcaccaccgagaag	5′-ggaagcaaattagcaagattgga
TaLEA1	AY148490	5′-caccaacaccaccaaggaca	5′-cgccaacacatgcgtctagt
TaAPX1	HX167147	5′-accctgctgactgaccctgt	5′-cctgaggtgtgcctccttgt
TaAPX2	HX177140	5′-gaccgacaagaccctcctga	5′-catcctcatccgcagcatatt
TaFSD2	HX176689	5′-tcgtgtcatgggaaatggtg	5′-gttgcttccttcgctgcttc
TaGA3ox2	AK330302	5′-gtcacgtggcccgagtacat	5′-catcgttgtcggtggagatg
TaGIP	EU095332	5′-tgcccctgctacaacaactg	5′-tggcaccccagaagaagaag
TaSLRL1	HG670306*	5′-acggcgaagataaccataccag	5′-cccgctacacttaccgttgc
TaExpA4	AY543530	5′-gagcaggaactggggtgcta	5′-gtccgtcgtcggtctgtacc
TaExpB1	CJ803265	5′-ggcacgtcctacagctcctc	5′-gctcacaaattgcagaaccaga
TaRP15	HX098404	5’-gcacacgtgctttgcagataag	5′-gccctcaagctcaaccataact

The full-length sequences of some genes were obtained by extending partial EST sequences from the NCBI wheat EST database (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and wheat genome sequence databases (http://www.cerealsdb.uk.net/cerealgenomics/CerealsDB/search_reads.php and https://urgi.versailles.inra.fr/blast/blast.php). Some primers match with three homoeologous genes, as *T. aetivum* is a hexaploid species.

* Sequence position in HG670306: 425714967-425716472

Supplementary Table S2. Summary of the DNA-binding specificity of TaZFP46.

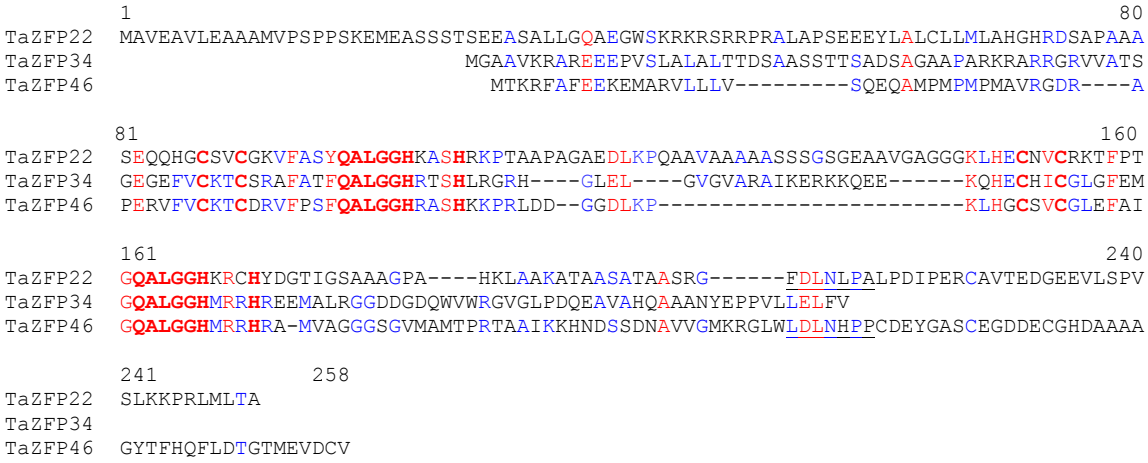
Binding affinity	Left sequence	Spacer length between two SAGTR motifs	Right sequence	Relative binding activity to preferred base
Preferred	GG**GAGTG**A	N8	GG**GAGTG**A	100%
High	AC	N8	AC	71-90%
Medium	TT AB	N5-11	TT AB	41-70%
Low	HC	N5-11	HC	20-40%

Data are based on Figure 2. The preferred binding sequence of TaZFP46 is GG**GAGTG**AN5GG**GAGTG**A. H = A, C or T; B = C, G or T; S = C or G; R = A or G.

Supplementary Table S3. Expression levels of *TaZFP34* and potential root growth-related, ABA signaling pathway or antioxidant enzyme genes in the roots of wild-type and transgenic plants expressing high-level *TaZFP34*.

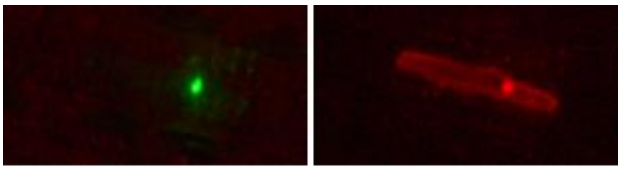
Gene name	Gene description	ZFP34-2	ZFP34 -13	Wild type	Ratio T/W†
TaZFP34		0.042 ± 0.014**	0.049 ± 0.006**	0.001 ± 0.000	**46**
Root growth-related genes					
TaRR12B	Two-component response regulator	0.017 ± 0.004*	0.018 ± 0.001*	0.033 ± 0.009	**0.53**
TaRR12D	Two-component response regulator	0.041 ± 0.013*	0.051 ± 0.017*	0.099 ± 0.008	**0.46**
TaRR1L1	Two-component response regulator 1-like	0.870 ± 0.073	0.768 ± 0.033*	1.089 ± 0.210	**0.75**
TaRR1L2	Two-component response regulator 1-like	0.244 ± 0.029	0.241 ± 0.044	0.257 ± 0.032	0.94
TaIAA7	Aux/IAA protein	2.62 ± 0.42*	2.71 ± 0.19*	3.59 ± 0.33	**0.74**
TaIAA8	Aux/IAA protein	1.43 ± 0.15	1.27 ± 0.07*	1.47 ± 0.04	**0.92**
TaIAA27	Aux/IAA protein	0.114 ± 0.006*	0.116 ± 0.005*	0.143 ± 0.013	**0.80**
TaSHY2	Aux/IAA protein	0.977 ± 0.061*	0.933 ± 0.031*	1.322 ± 0.203	**0.72**
TaGA20-ox1	Gibberellin 20-oxidase	0.030 ± 0.008*	0.040 ± 0.004*	0.062 ± 0.011	**0.56**
TaGID1	Gibberellin receptor	0.933 ± 0.148*	0.934 ± 0.110*	0.632 ± 0.067	**1.48**
TaRht1	DELLA protein	0.239 ± 0.027	0.213 ± 0.024	0.286 ± 0.049	0.79
TaARF1	Auxin response factor	0.187 ± 0.010	0.198 ± 0.016	0.193 ± 0.018	1.00
TaARF2a	Auxin response factor	0.061 ± 0.003	0.065 ± 0.004	0.062 ± 0.003	1.02
TaARF2b	Auxin response factor	0.160 ± 0.005	0.151 ± 0.010	0.140 ± 0.022	1.11
TaARF6	Auxin response factor	0.013 ± 0.004	0.017 ± 0.003	0.017 ± 0.004	0.88
TaETTINa	ETTIN-like auxin response factor	0.038 ± 0.004	0.034 ± 0.005	0.038 ± 0.001	0.95
TaETTINb	ETTIN auxin response factor	0.051 ± 0.006	0.047 ± 0.004	0.048 ± 0.004	1.02
TaTIR1L1	Transport inhibitor response 1-like	0.313 ± 0.035	0.266 ± 0.028	0.308 ± 0.038	0.94
TaTIR1L2	Transport inhibitor response 1-like	0.674 ± 0.052	0.558 ± 0.024	0.593 ± 0.007	1.04
TaTIR1L3	Transport inhibitor response 1-like	0.259 ± 0.012	0.181 ± 0.018	0.208 ± 0.038	1.06
TaPIN1a	Auxin efflux carrier protein	0.0017 ± 0.0002	0.0013 ± 0.0001	0.0013 ± 0.0004	1.15
TaPIN1b	Auxin efflux carrier protein	0.155 ± 0.008	0.175 ± 0.005	0.215 ± 0.042	0.77
TaPIN2	Auxin efflux carrier protein	0.282 ± 0.019	0.295 ± 0.030	0.293 ± 0.041	0.99
TaPIN4	Auxin efflux carrier protein	0.117 ± 0.028	0.082 ± 0.021	0.083 ± 0.023	1.20
TaExpB18	Expansin B	0.009 ± 0.003*	0.012 ± 0.002	0.017 ± 0.003	**0.62**
TaCYCB1-2	Cyclin B1	0.073 ± 0.025	0.087 ± 0.021	0.089 ± 0.022	0.90
TaCYCB2-3	Cyclin B2	0.050 ± 0.015	0.054 ± 0.016	0.058 ± 0.011	0.90
TaCYCD4-1	Cyclin D4	0.171 ± 0.012	0.174 ± 0.015	0.175 ± 0.030	0.99
ABA metabolic, signalling or drought-upregulated genes					
TaNCED3	9-cis-epoxycarotenoid dioxygenase 3	0.009 ± 0.001	0.014 ± 0.002	0.009 ± 0.003	1.28
TaNCED9	9-cis-epoxycarotenoid dioxygenase 9	0.015 ± 0.001	0.019 ± 0.001	0.017 ± 0.001	1.00
TaCYP707A1	ABA 8'-hydroxylase 1	0.375 ± 0.027	0.401 ± 0.040	0.359 ± 0.056	1.08
TaABI1	Protein phosphatase 2C	0.209 ± 0.005	0.228 ± 0.012	0.260 ± 0.037	0.84
TaNAC69-1	ABA-inducible NAC transcription factor	0.084 ± 0.005	0.055 ± 0.005	0.070 ± 0.013	0.99
TaWRAB1	Late embryogenesis abundant protein	0.161 ± 0.028	0.121 ± 0.043	0.180 ± 0.025	0.78
TaWRAB18	Late embryogenesis abundant protein	0.291 ± 0.193	0.499 ± 0.054	0.488 ± 0.072	0.81
TaLEA1	Late embryogenesis abundant protein	0.027 ± 0.006	0.019 ± 0.009	0.021 ± 0.002	1.10
Antioxidant enzyme genes					
TaAPX1	L-ascorbate peroxidase 1	8.96 ± 0.17*	10.11 ± 0.65	13.35 ± 2.41	**0.71**
TaAPX2	L-ascorbate peroxidase 2	17.27 ± 0.41*	23.65 ± 1.11*	29.90 ± 1.42	**0.68**
TaFSD2	Fe-superoxide dismutase 2	0.081 ± 0.001	0.104 ± 0.008	0.108 ± 0.020	0.86

The root samples were from 3-week-old hydroponically grown wild type and T_2 transgenic (ZFP34-2 and ZFF34-13) plants. Values are means ± SD of 3 biological replicates and expression levels are relative to an internal reference gene, *TaRP15*. GenBank accession numbers of these genes are shown in Supplementary Table S1. Values in bold are those with a significant difference at least in one of the two transgenic lines.
* $P < 0.05$ using Student's *t*-test; ** $P < 0.01$;
† Ratio of the mean values of two transgenic lines to that of the wild type control.

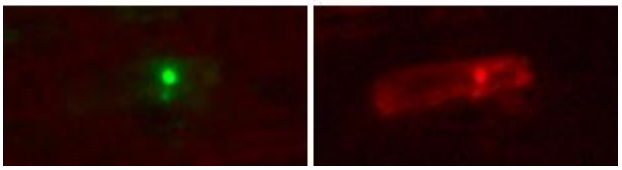


Supplementary Figure S1. Amino acid sequence alignment of TaZFP22, TaZFP34 and TaZFP46. C_2H_2 residues and QALGGH motifs are in bold. EAR motifs are underlined.

Ubi1GFPZFP22



Ubi1GFPZFP46



Supplementary Figure S2. Subcellular localisation of TaZFP22-GFP and TaZFP46-GFP fusion proteins in wheat leaves. Each TaZFP-GFP construct (Ubi1GFPZFP22 or Ubi1GFPZFP46) was co-bombarded with an Act1RFP construct. The RFP red fluorescence illustrates the shape of transformed leaf epidermal cells (shown at the right) in the RFP channel. These N-terminal GFP-fused TaZFP22 and TaZFP46 proteins were localised in the nucleus.

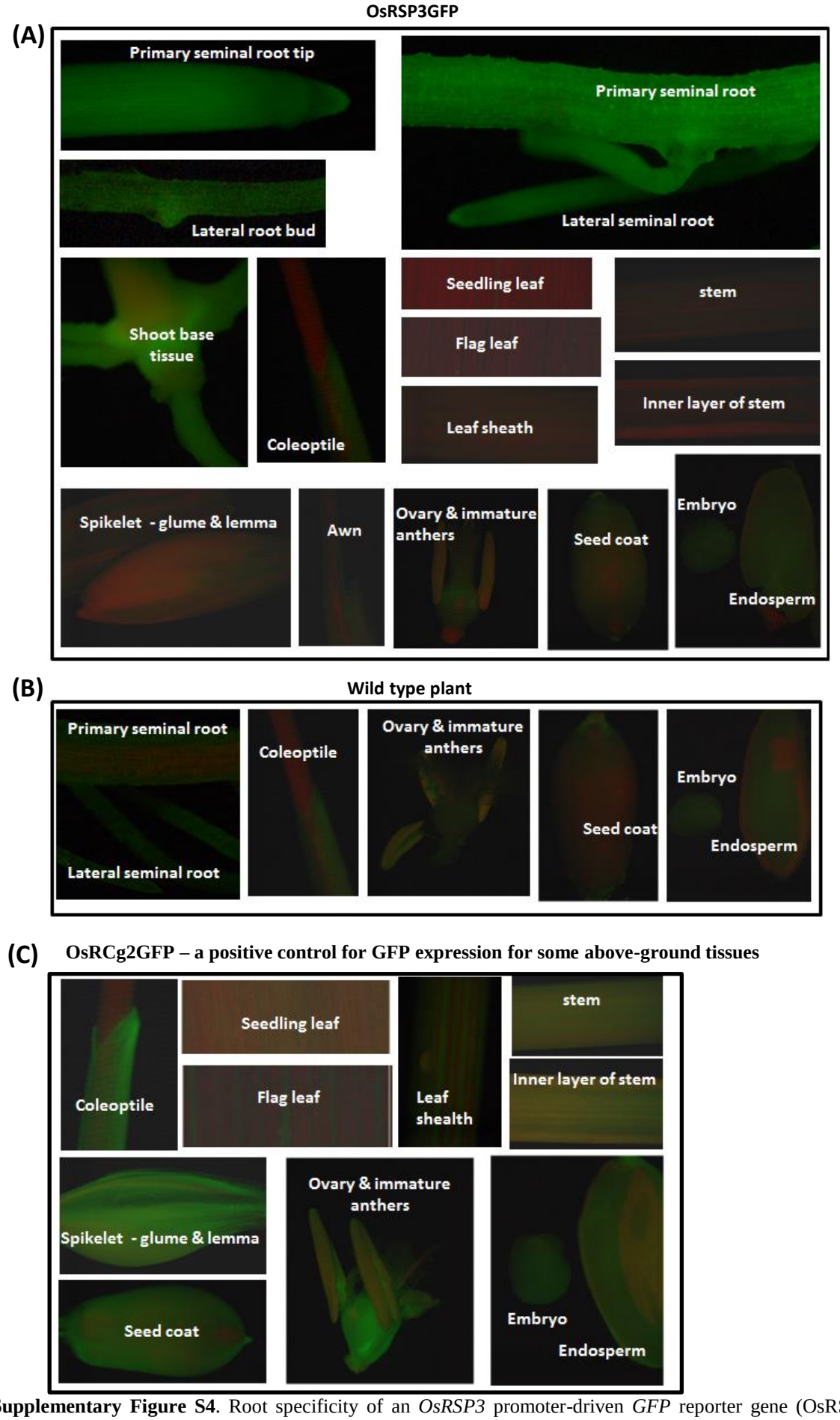
A

ZFP46S1	**CAGTA**AGCTGA**CAGTG**ACGGAA**CACTG**CCTA
ZFP46S2	CT**GAGTG**AGAAG**GAGTG**CAATCACAAATGGC
ZFP46S3	GCACATG**GAGTG**GATGTTA**CAGTG**CTTGAAT
ZFP46S4	CTGAG**GAGTG**ATTCAAT**CACTC**CCATCCAAGC
ZFP46S5	**CACTC**ATATA**CAGTA**TGCGAT**TACTC**CTGA
ZFP46S6	ATTCCAG**CAGTG**TGAATGT**CAGTG**CGATTGT
ZFP46S7	G**GAGTG**CTGAACT**GAGTG**CGTACACAAGCAT
ZFP46S8	**CACTC**TAGCACA**CAGTA**TCAAATGG**CAGTA**TC
ZFP46S9	**CACTC**AAGATT**GAGTG**TGCGTATAG**CACTC**CA
ZFP46S10	GCACCATGG**GAGTG**GATTTGA**CAGTG**GAATTGT
ZFP46S11	CCAAGCTCTGTGA**CAGTG**ACATTAG**GAGTG**CTCG
ZFP46S12	CGCTGAG**GAGTG**ATTCACA**CAGTG**CCCCATAA
Consensus	SAGTR

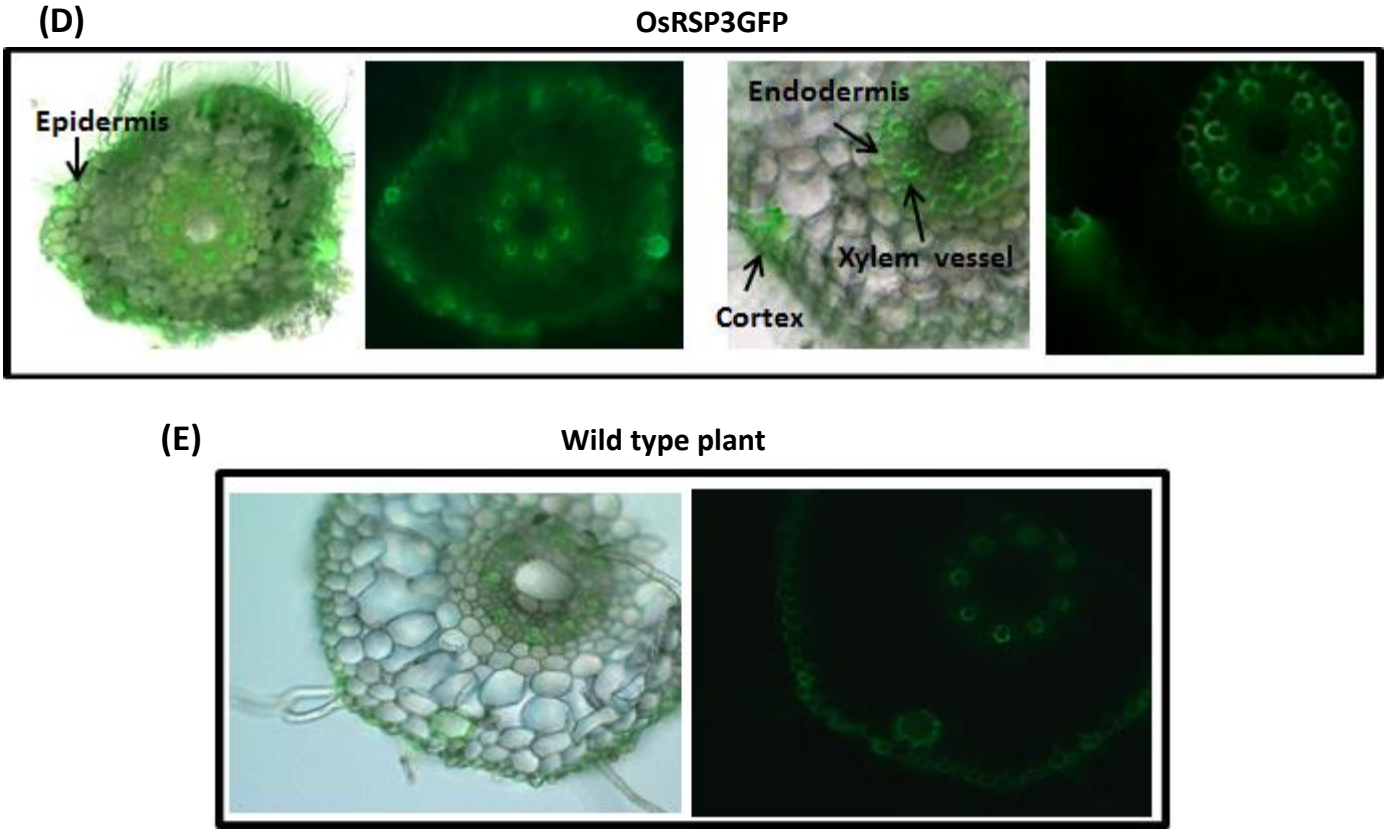
B

Scanning core region		**RBA**
EP1	GGTTGA**CAGTG**TCACATGA**CAGTG**TCATT	1.00 ± 0.07
EP1m1	GGTaGA**CAGTG**TCACAaGA**CAGTG**TCATT	0.97 ± 0.11
EP1m2	GGTTaA**CAGTG**TCACATaA**CAGTG**TCATT	0.46 ± 0.12
EP1m3	GGTTGc**CAGTG**TCACATCc**CAGTG**TCATT	0.52 ± 0.04
EP1m4	GGTTGAa**AGTG**TCACATGAa**AGTG**TCATT	0.17
EP1m5	GGTTGA**C**c**GTG**TCACATGA**C**c**GTG**TCATT	0
EP1m6	GGTTGA**CA**a**TG**TCACATGA**CA**a**TG**TCATT	0
EP1m7	GGTTGA**CAG**a**G**TCACATGA**CAG**a**G**TCATT	0
EP1m8	GGTTGA**CAGT**aTCACATGA**CAGT**aTCATT	0.30 ± 0.05
EP1m9	GGTTGA**CAGTG**aCACATGA**CAGTG**aCATT	1.91 ± 0.17
EP1m10	GGTTGA**CAGTG**TtACATGA**CAGTG**TtATT	0.76 ± 0.15
EP1m11	GGTTGA**CAGTG**aaACATGA**CAGTG**aaATT	1.01 ± 0.09
EP1m12	GGTTGAt**AGTG**aCACATGAt**AGTG**aCATT	1.23 ± 0.16
EP1m13	GGTTGAg**AGTG**aCACATGAg**AGTG**aCATT	**2.43 ± 0.23**
EP1m14	GGTTGA**CAG**a**G**aCACATGA**CAG**a**G**aCATT	0
EP1m15	GGTTGA**CAG**c**G**aCACATGA**CAG**c**G**aCATT	0.09 ± 0.02
Defining sequences flanking the core		
EP1m13	GGTTGAg**AGTG**aCACATGAg**AGTG**aCATT	2.43 ± 0.23
EP1m16	GGTTGAg**AGT**aaCACATGAg**AGT**aaCATT	0.80 ± 0.17
EP1m17	GGTTGAg**AGT**caCACATGAg**AGT**caCATT	0.05 ± 0.01
EP1m18	GGTTGAg**AGT**taCACATGAg**AGT**taCATT	0.22 ± 0.03
EP1m19	GGTTGAg**AGTG**cCACATGAg**AGTG**cCATT	1.13 ± 0.13
EP1m20	GGTTGAg**AGTG**gCACATGAg**AGTG**gCATT	1.41 ± 0.15
EP1m21	GGTTGAg**AGTG**TCACATGAg**AGTG**TCATT	1.27 ± 0.08
EP1m22	GGTTGcg**AGTG**aCACATCcg**AGTG**aCATT	0.92 ± 0.06
EP1m23	GGTTGgg**AGTG**aCACATGgg**AGTG**aCATT	**3.07 ± 0.28**
EP1m24	GGTTGtg**AGTG**aCACATGtg**AGTG**aCATT	1.71 ± 0.16
EP1m25	GGTTAAg**AGTG**aCACATaAg**AGTG**aCATT	0.58 ± 0.08
EP1m26	GGTTcAg**AGTG**aCACATcAg**AGTG**aCATT	0.79 ± 0.10
EP1m27	GGTTtAg**AGTG**aCACATtAg**AGTG**ACATT	1.05 ± 0.03
Defining spacer length		
EP1m23	GGTTGgg**AGTG**aCACATGgg**AGTG**aCATT	3.07 ± 0.28
EP1m28	GGTTGgg**AGTG**aCACaATGgg**AGTG**aCATT	1.91 ± 0.02
EP1m29	GGTTGgg**AGTG**aCACaaATGgg**AGTG**aCATT	1.37 ± 0.07
EP1m30	GGTTGgg**AGTG**aCACaaaATGgg**AGTG**aCATT	1.33 ± 0.09
EP1m31	GGTTGgg**AGTG**aCACaaaaATGgg**AGTG**aCATT	0.52 ± 0.03
EP1m32	GGTTGgg**AGTG**aCACaaaaaATGgg**AGTG**aCATT	0.49 ± 0.08
EP1m33	GGTTGgg**AGTG**aCAATGgg**AGTG**aCATT	1.53 ± 0.04
EP1m34	GGTTGgg**AGTG**aCATGgg**AGTG**aCATT	1.68 ± 0.09
EP1m35	GGTTGgg**AGTG**aCTGgg**AGTG**aCATT	1.92 ± 0.10
EP1m36	GGTTGgg**AGTG**aCGgg**AGTG**aCATT	0.49 ± 0.04
EP1m37	GGTTGgg**AGTG**aGgg**AGTG**aCATT	0.52 ± 0.10

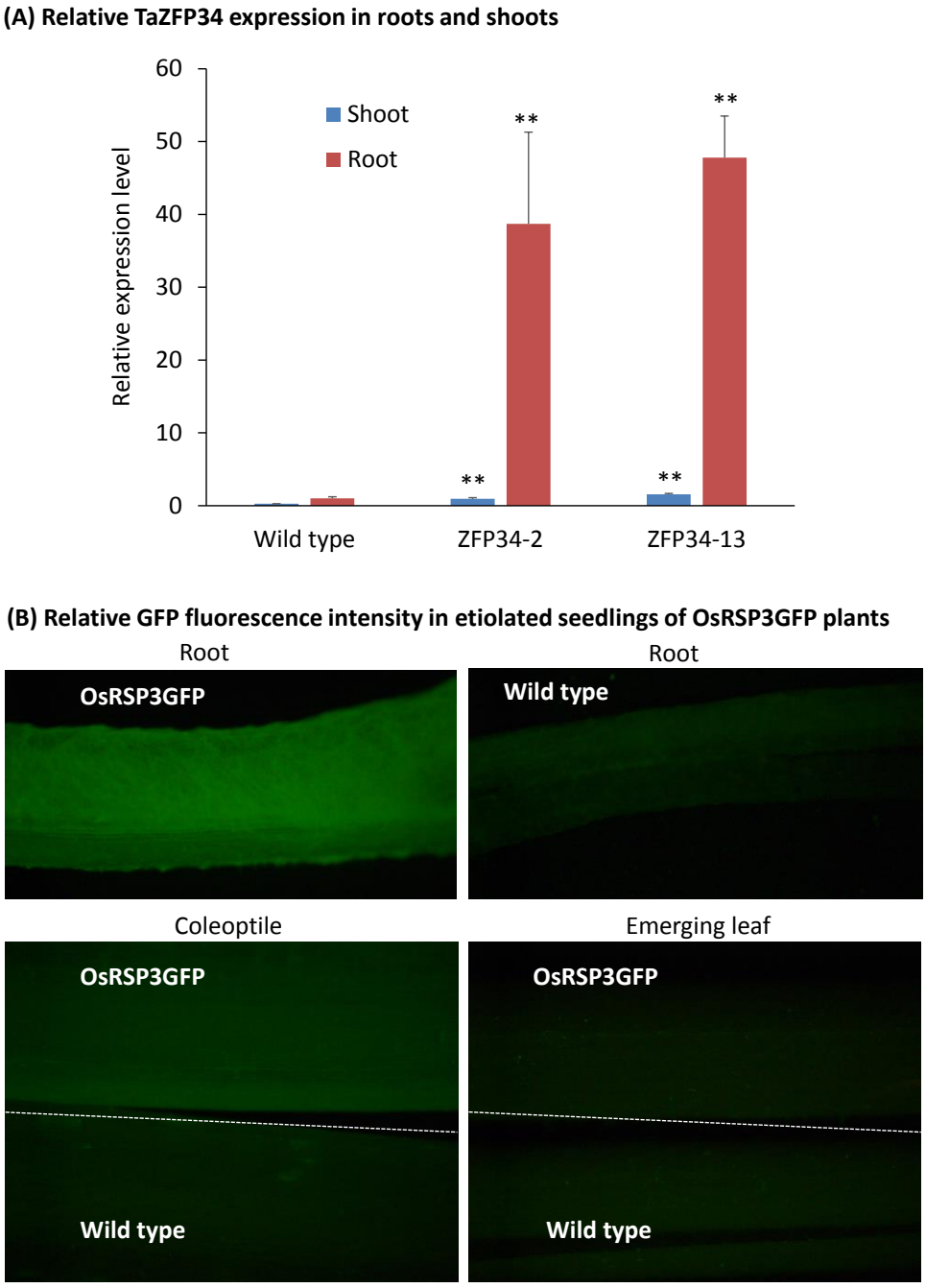
Supplementary Figure S3. The DNA-binding specificity of TaZFP46
(A) *In vitro* selected DNA-binding sites of TaZFP46. Spacer length between SAGTR (or YACTS) and SAGTR is 5-9 nucleotides. S = C or G; R = A or G; Y = C or T.
(B) Systematic base substitution and insertion/deletion analysis of the TaZFP46 binding sequence using the EP1 element as a starting motif. Values are means ± SD of 2-3 assays. Binding activity is expressed as relative to that of EP1. SAGTR in EP1 is typed in bold letters and substituted or inserted bases to EP1 in lower-case letters. Values with a marked increase in binding activity are in bold. RBA, relative binding activity of TaZFP46.



Supplementary Figure S4. Root specificity of an *OsRSP3* promoter-driven *GFP* reporter gene (OsRSP3GFP) in transgenic wheat plants. Root specificity of OsRSP3GFP reporter in transgenic wheat plants is illustrated in (**A**) with wild type control in (**B**) and OsRCg2GFP (Xue et al., 2016, Reference # 38) as a positive control for some above –ground tissues (**C**).



Supplementary Figure S4-continue. Cell specificity of OsRSP3GFP reporter expression is shown in (**D**) with wild type control in (**E**). Cross sections of wheat primary seminal roots (**D** & **E**) are shown as merged images of a bright-field image and a GFP fluorescence image with corresponding GFP fluorescence images placed at the right side. Low green auto-fluorescence background is present in wild type root cells (**E**).



Supplementary Figure S5. Relative TaZFP34 expression levels in the roots and shoots of two high *TaZFP34*-expressing transgenic lines and relative GFP fluorescence intensity in the etiolated seedlings of OsRSP3GFP plants.
(A) Relative *TaZFP34* expression levels in the roots and shoots of two high *TaZFP34*-expressing transgenic lines (ZFP34-2 and ZFP34-13). Three-week-old seedlings were used for expression analysis. Values are means + SD of 3-4 biological replicates and expression levels are expressed as relative to that in the roots of the wild type plants. Statistical significance of differences between control and transgenic lines is indicated by asterisks (** $P < 0.01$).
(B) Relative GFP fluorescence intensity in the etiolated seedlings of OsRSP3GFP plants. One-week-old dark-grown seedlings were used for analysis.

TaRR12D promoter

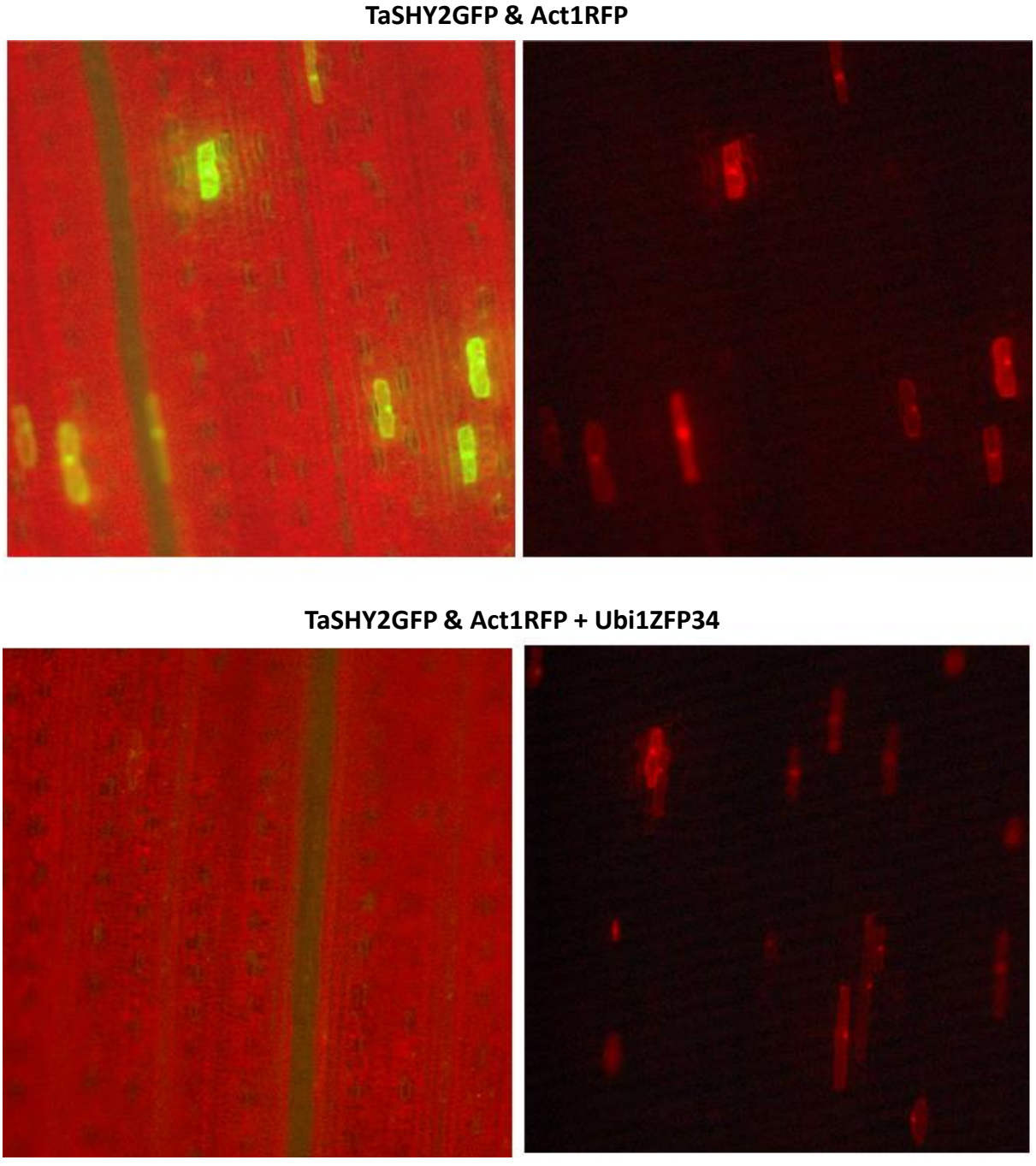
CTTGTGGGGTTGAGGTTGTACTGCATGCACGGTGGAGGGGTGCAACGGCCAAGAGAACGAGCAGAACGCCAAGCCGAATTCATACCTTAAGGATGGTACGCGCGAAGAG
ATGGGATCGCCCGCCGCCGTCGCCGCCGATTCAGCCGGCGCAGATCCAGCCTTCTTCTTCTTCGGTGACGGCGTGGGGGCTGGGGTTGGTTGGGTGGAGGACGAGGACG
ATGAATTTATTCCTCTCACCAGGCAGGTCGAGGACGGAGAAGGTCAGGAGCGGCGAGCGGCGACGAATACATCGGAGGAGGATTCTGAACTGGATCGAGTAGACATGGA
TCTGGTCCTCAGGTGATCGGTTCAAGAAAAGATATTTTCCCCTGGACCATTATGCCCTTATCGCAATTAACATATTAAATTTAATCAATTAAAATTCCCCGCAAGATCG
GACGGTTAATAAAAATCAGACGTGATCAGACATGTAAGTACTGCTAAGTACTCCAAGTACTAACCCAATCACCATAAAACAGCTAATTATATATACTGTGTTGAGCCCA
RR12DBS1
AAATAGCACTCGGCGAGCAGAGAGCACCTTTCAATGGTATACTACAAGACAATCTACAAAAATATTAAGAAATTGCTTCAAACGTTCATGGCTTTTTGTAGTTGAATTC
AAACTGCAGAAACTTCTTACGTTAGTTTCCAGAGGGAGTAGCATATATTCTCTCTCTCTCTCTCTCTCACATCACCATCCTGCATTTTTTATCTCAGTTCATCCCACAG
RR12DBS3
TAAATGTGAAAAGAAAAAACAATGCTGCAGAAATCAAATCACCAGGAATGGAGAAACAACCGGAGCAAGCAAGCAAGCAGCCCCCAACTGCCATGCCATTGGAGGGTTG
TGGCAGGTGGGTCCCACGGGCTCCTCCTCCTGTAGCCTTGCACCATATGCAAACGCAAACGCAAACGCAGCGAGCACTTCCACTCTGATGACTGACCCCTATAAATCGG
RR12DBS2
CCACCAGCAGTCCAAAACTTTGCTTTCCATCGCCATCGAAACCTGCTCGCCCTTTCACCTCCCCAAACAACACCCTCCCCACCTCACCAAGCGTGCTGCTGCTGCTGCG
GTGGTTTGGCTGCTTCCTCCTCCTCCTCCACCTCGTCGTCGTCGTCTTCTACCTTTGCCTGCGTCGGTTGCTTGCTGTGGTGCGGTGTGGCTGGGCTGGGCTGCGAGCA
CGGGCAGGCAGGGCTTGCTTGCTCGCTTGGGCACCCGGAGTGTCGCTGTCTGTCTGTCTCTGCCTTTCTTCTTTCCCCTCTCTGTTTTGTGTCCTTGCTCTCCTTTGGC
CTTTTGCTTTCCCAACCTCTCCCTTCCCGGCCTCAATAATGATGGAGGGGCCGCGGCGGGTTCGTTCCTGAAGGCCTTCTCCCCCCGTCCCGTCCATTCTCGTCTCAAT
CTCCACCGGTGGGCCGGAATACAAATCCACCGGCAGTTTTCCCCTCTCTGATCTTCTCTCTTCCCGGAGTAATTTTGGTTCTAGGTTGTTTCTTTGGGGCCTTCCCTTC
TGTTGTGTTCAGGTGGTGTTGGTGTCGAGCGCTCGATTAAAGTCCCCTTTCTTCTTCCTCTTAATTGCTAGCTGCCATTAGCTTCTCAGGGTTCTTGCTGGGGATTGGG
TCAATTGGGAGCTTTCGTCTTTAGGGTCAATTGGG**ATG**

TaSHY2 promoter

AAATCGCACTAGTTCAACCGTTGGGTTTTTTTACCATGAATTTACATTGCATAAATGCACGAAAACTTTGTTTAATCTTGCAAAAAGAGATTATTTTACTAAATAATAA
ATAAGTTTTTTTCCCAGGATTTCCTCAAGCTGAGCCCGGCATTCCTGATATCCCGTAGGCCAAATTCATCTTGTTTGATAATCTAGCTGAAGCTAGGGACCACCTTTGC
TCACCAGGAAAATGAATATACAAGCATCGGTTAGTTAAAGGAGCAGCACCATCACTGCAGTTCACAATACTTTGCAGTTTCCATAGAAAAGAAAAGGTGAAGTGGTTAA
SHY2BS1 SHY2BS2
CAGTGCACTTTGCAAGGGAACTTTTGGACTAATTAAACAAGCTCCCCTACCACTAGGGCCTGCTTTGCATAGGCAAAAGAAGCAAGGGCAGGGAAAAGAAGAAACAAAA
GGTAGTGGAGAGAGATGCAGGTATATGATGAGGAGCATGCAATAGACAAGTGCCCATATACTCCATGTTACATTGTTCCATACATATCTTATTATTGGGGAGGAGGGAG
GAGACAGTTTTGTATTAGCCAAAGATGCTCCCCCAAGATCTCTAATGATAACATCCTAATCATGGCATTGGCGCCCTATTTATTTTATTTATTTAATCAAGCTAAATTT
CATCAGTGCTGTTTGAACCTTTCTAGATGACGGGTACCGGTGCCGGGCCCAGATCCATGCCCGGTTCAACCGGGTTATGGTAACTACATTTTGAGGATAATTGATATGG
GGTCCAATTAGTAATGTACACGTACAGTGATCACGTACCGGGCTATCGGGTCAAGTGCAGCAATCCAGCAACGACTCGACCCGCCGGCACCCGAGAATCTCTAGTGGTA
CGCCTAGTGGAGCCGCGTGGGTCCCGCCGGGTCGGCCCGCCCTGTCGCGGGTCGCCGCCGGCAAGGCCCCCATGGCTGCACCCCACCCCGCAACCACCCCAGCTACCTG
TGGTAAGTGTGTGGACGCCGCACATGCGCGTGGCCTGGGCTGTGGCACGTACGTGTGCGTGCATGTGGACGCATGTGGAGTTGGAGCCTCCCTCACACGGGCGCGCCAC
ACGCCCGCCTATCTCTCCCTCTCCTCTCCTCGTCGCAGCAGGCCTACCACCCGGGGGCTCCGCTCTATAAAACATTTCCATGGAGAACCTCCACAGCTCACAAACCCTCA
CCACTCACCTACAGCCAGCTGAGAAGAACGACTTGCAAGTCCTAACCACCTCTAGTCGGTAGACACGCACCTCGTCGCCGGAGACAGGAACTGATG

Supplementary Figure S6. Promoter region sequences of *TaRR12D* and *TaSHY2*. The underlined ATG is a translation initiation codon. SAGTR (or YACTS)-like motifs containing sequences used for DNA-binding assays are highlighted.

TaRR12D and *TaSHY2* promoter sequences were isolated from wheat genomic DNA (genotype: SB169) using PCR-amplification, based on the wheat genome sequence database in URGI (https://urgi.versailles.inra.fr/blast/blastresult.php?jobid=141834266111&opt=none). The following two sequence IDs used for promoter isolation are WGSC_chr7DS_ab_k71_contigs_longerthan_200_3948850 (the *TaRR12D* promoter) and IWGSC_chr5DS_ab_k71_contigs_longerthan_200_273208 (the *TaSHY2* promoter). Primers used were: 5'-cacagatcttgtggggttgaggttgtac (sense primer) and 5'-ctgccatggcaattgaccctaaagacgaaag (antisense primer) for the *TaRR12D* promoter; 5'-ccggaattcaaatcgcactagttcaaccg (sense primer) and 5'-cgcggatccacgaggtgcgtgtctaccgact (antisense primer) for the *TaSHY2* promoter. The underlined sequences are restriction enzyme sites used for cloning. The PCR-amplified DNA fragments were cloned and sequenced.



Supplementary Figure S7. Illustration of co-transformation efficiency of TaSHY2GFP and Act1RFP constructs in wheat leaves using particle bombardment-mediated transformation and repression of TaSHY2GFP expression by *ZmUbi1* promoter-driven TaZFP34 (Ubi1ZFP34). Left panels are GFP channel images and right panels are corresponding RFP channel images. This illustrates that the co-transformation efficiency of GFP and RFP constructs is quite high in this transient expression system.